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Biological Determination of Activities of Administered Antituberculous Drugs in the Blood of Animals

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Introduction

Since Kirchner[1] reported on the serum-synthetic medium in 1932, many investigations on suitable concentrations of serum for the growth of tubercle bacilli have been made, and the ten per cent[1),(2),(3),(4)] was largely recommended as the most suitable concentration, while it was less frequently reported that concentrations varying from 3 to 12 per cent[5] or 8 to 50 per cent[6] did not present any remarkable difference in the growth of bacilli.

On the other hand, it is naturally supposable that blood, especially serum acts as the first medium for the antituberculous substance absorbed into body, and when the bacilli meet the drug in the lesion, the medium may be also something similar to the serum.

The writers have found no report concerning the culture medium prepared so as to contain the serum in very high concentration in order to determine the bacteriostatic activities of chemotherapeutic agents *in vitro* under a condition as similar as possible to the medium in the body where the bacteriostatic action is performed.

This may be due to the presence of Slide Cell Culture Method which has been widely used for the same purpose up to this time, but it is believed that this method has a fault due to the fact that it presents considerable discrepancies in the results according to the techniques.

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In the present studies an increase in concentration of the serum to be contained in Kirchner's medium without hindering the growth of tubercle bacilli was tried, and as preferable results were obtained, a quantitative and successive determination of the bacteriostatic activities of the administered antituberculous drugs in the blood of the living body was progressively undertaken with the ordinary test tube growth using Kirchner's serum-synthetic medium.

First Experimental

(1) Culture Medium:

The medium prepared by adding one part of Kirchner's basal synthetic solution which is modified by using only one-tenth of the volume of distilled water that is used in the normal solution to nine parts of serum collected aseptically from rabbit, goat or human and sterilized at 56°C. water bath for 60 minutes was standardized in this experiment.

(2) Results and Discussion:

The comparison of the growth of bacilli in the above stated medium with that in unmodified Kirchner's media prepared as the control in which the serum was contained in concentration of 10 per cent may be seen in table 1.

Table 1:

Concentration of serum in medium	Amount of synthetic solution	Amount of added serum	Weeks after inoculation of bacilli				Dry weight of obtained bacilli
			1	2	3	4	
90 per cent	0.2 cc.	1.8 cc.	−	+	++	++	2.0 mg.
			−	+	++	++	2.1 mg.
10 per cent	1.8 cc.	0.2 cc.	−	+	++	++	1.7 mg.
			+	+	++	++	1.8 mg.

Explanation of symbols:

+ : Growth of bacilli only at the bottom of tube.

++ : Growth of bacilli both at the bottom of tube and on the surface of medium.

− : No growth of bacilli.

Two ml. of the medium was poured in each of 5 centrifuge tubes, and incubated after inoculation of bacilli. The presence and the amount of growth of bacilli were read at the end of the 1st, 2nd, 3rd and 4th weeks.

Kirchner's media containing 90 per cent serum were inferior to that with 10 per cent serum in the growth of bacilli at the bottoms of the tubes up to the end of the 2nd week, after which the growth of bacilli on the surfaces of Kirchner's

media containg 90 per cent serum became faster than that on the surfaces of the media with 10 per cent serum.

At the end of the 4th week the supernatant fluid was poured off after centrifuging at 3,000 rpm for 15 minutes and the average of the amounts of the dried sediment in every tube was measured. The results indicated that the amount of tubercle bacilli in the media containing 90 per cent serum was slightly larger than that in the media with 10 per cent serum.

Although Kirchner's medium with 10 per cent serum has been hitherto considered most suitable for the growth of bacilli, it was proved in the present studies that the medium with 90 per cent serum is not inferior, if the composition of the basal synthetic solution is modified suitably.

Second Experiment

As it was confirmed from the results of the first experiment stated above that observable growth of bacilli could be obtained both at the bottoms and on the surfaces of 90 per cent serum-synthetic media, the antituberculous activities of the drugs were investigated, employing this medium, and the results may be seen in table 2.

Table 2:

Tested substance	Concentration of serum in medium	Concentrations of drug							Control
		2.5×10^4	5×10^4	10^5	2×10^5	4×10^5	8×10^5	16×10^5	
Conteben	90 per cent	−	+	+	+	−	/	/	+
	10 per cent	−	+	+	+	+	/	/	+
	90 per cent	−	+	+	+	+	/	/	+
	10 per cent	−	+	+	+	+	/	/	+
PAS	90 per cent	/	−	−	+	+	+	+	+
	10 per cent	/	−	−	−	+	+	+	+
	90 per cent	/	−	−	+	+	+	+	+
	10 per cent	/	−	−	−	+	+	+	+
St.-mycin	90 per cent	/	/	−	−	−	+	+	+
	10 per cent	/	/	−	−	−	−	−	+
	90 per cent	/	/	−	−	+	+	+	+
	10 per cent	/	/	−	−	−	−	+	+

− : No growth of bacilli.
+ : Growth of bacilli was observed.
/ : Test was omitted.

(1) Materials:

Streptomycin, PAS and conteben were sterilized by leaving them in 70 per cent alcohol for 3 to 4 hours prior to being diluted to the definite concentrations with distilled water.

(2) Results and Discussion:

In comparing the bacteriostatic activities of streptomycin, PAS and conteben in the media containing 90 per cent serum with those in Kirchner's media containing 10 per cent serum, weaker activities of those drugs except conteben were indicated in the media with 90 per cent serum. The fall of bacteriostatic activity of streptomycin with the rise of serum concentration was remarkable while that of PAS was slight. This finding is agreeable with the report by Williston and Youmans[7] that the bacteriostatic activity of streptomycin declined when the serum concentration in the serum-synthetic medium was increased to nearly 30 per cent without modifying the basal synthetic solution.

It may be a desirable subject of future studies to investigate what character of the drug causes those results, and we, clinicians should consider this uncertain character as one of the factors which causes the discrepancy between the effects of the drugs *in vitro* and *in vivo*.

Third Experiment

(1) Method:

Normal rabbits weighing 2 to 3 kg. were injected intravenously in the ear vein with a solution of one of the drugs; blood was collected by puncturing the heart successively 30 minutes, 1 hour, 2 hours, 2-half hours, 3 hours, 4 hours and 24 hours after the injection, and was allowed to remain for 24 hours so as to form slants in test tubes in order to separate the serum; media containing 5, 10 and 90 per cent of this serum were prepared; bacilli were inoculated in each of these media; after being sealed with cotton and paraffin the cultures were incubated for 4 weeks, and then the growth of tubercle bacilli was read, being compared with that in the medium containing the serum obtained from the same animal before the injection of the drug.

Streptomycin and PAS in dose of 0.1 gm. per kg. in aqueous solution and conteben in dose of 0.01 gm. per kg. in 1 per cent solution of propylenglycol were employed as the drugs.

(2) Results and Discussion:

From the results of the experiments shown in tables 3, 4 and 5, the following may be drawn:

(1) It is demonstrated *in vitro* that streptomycin and PAS maintain their antituberculous activities in the serum of animals following injection of the drug.

(2) Antituberculous activity in the blood serum following intravenous administration of 0.1 gm. of streptomycin or PAS per kg. is retained for at least 2 and one-half hours. This result is agreeable with the report describing that the concentrations of streptomycin and PAS in the blood measured by a traditional quantitative determination method dropped remarkably 3 to 6 hours after intravenous administration of the drug, although certain individual variations were noted.

Table 3: The growth of tubercle bacilli in medium containing the serum of a rabbit in which streptomycin was injected

Body weight of used animal	Dose of strepto-mycin	Concent-ration of serum in medium	Hours from injection of drug to collection of blood							Control
			0.5	1.0	1.5	2.0	2.5	3.0	24.0	
2.4 kg.	2.4 cc. in 10%	5%	/	/	+	+	++	++	++	++
		10%	/	/	−	+	++	++	++	++
		90%	/	/	−	−	−	+	+	+
2.5 kg.	2.5 cc. in 10%	5%	−	−	−	+	++	/	/	++
		10%	−	−	−	−	+	/	/	++
		90%	#	−	−	−	−	/	/	+

Table 4: The growth of tubercle baclli in medium containing the serum of a rabbit in which PAS was injected

Body weight of used animal	Concent-ration of serum in medium	Hours from injection of drug to collection of blood								Control
		0.5	1.0	1.5	2.0	2.5	3.0	4.0	24.0	
3.0 kg	10%	−	−	/	/	/	/	+	++	++
	90%	−	−	/	/	/	/	+	++	++
3.2 kg.	5%	/	−	/	−	/	/	+	++	#
	10%	/	−	/	−	/	/	+	++	++
	90%	/	#	/	#	/	/	−	+	+
3.0 kg.	5%	/	/	−	−	++	++	/	/	++
	10%	/	/	−	+	++	++	/	/	++
	90%	/	/	−	−	−	+	/	/	+

Table 5: The growth of tubercle bacilli in medium containing the serum of a rabbit in which conteben was injected

Concentration of serum in medium	Hours from injection of drug to collection of blood								Control
	0.5	1.0	2.0	3.0	4.0	6.0	24.0	56.0	
5 per cent	++	++	++	/	/	/	/	/	++
10 per cent	++	++	++	/	/	/	/	/	++
90 per cent	−	−	−	/	/	/	/	/	++
5 per cent	/	/	/	++	++	++	++	++	++
10 per cent	/	/	/	++	++	++	++	++	++
90 per cent	/	/	/	−	+	++	++	++	+

(1) Streptomycin and PAS were administered in dose of 0.1 gm. per kg. into their own individual test rabbit.

(2) Explanation of symbols:

− = No growth of bacilli

+ = Growth of tubercle bacilli only at the bottom of tube
++ = Growth of tubercle bacilli both at the bottom and on the sulface of medium.
/ = Test was omitted
= Growth of saprophytes

(3) Conteben was administered in dose of 0.01 gm. per kg. in propylenglycol solution.

According to these results, there is no difference between the durations of antituberculous activities of streptomycin and PAS.

Considering the variations of the clinical doses and the effects of those drugs, it is believed that the mechanisms of the antituberculous chemical drugs are quite complicated. On the other hand, absence of remarkable variation between the durations of measurable concentrations of PAS and streptomycin in the blood leads the writers to think that intravenous administration of PAS will be worth investigating further.

(3) The relation between the duration of bacteriostatic activity and the variation of the serum concentration in the culture media is worth observing. For example, the same amount of bacteriostatic activity of streptomycin which is present in the medium containing 90 per cent serum collected within 2 and one-half hours after injection can be demonstrated in the medium with 5 per cent serum obtained not later than 1 hour after injection. This suggests that the bacteriostatic activity in the serum 1 hour after injection of the drug is approximately twenty times as strong as that 2 and one-half hours after injection. Thus, the results of the present study suggests the strength of the bacteriostatic activity immediately after injection and also considerable rapid fall of the activity.

(4) Due to the insolubility of conteben in water, propylenglycol was used as solvent and only 0.01 gm. of conteben per kg. was administered. Because of this fact, this drug connot be compared with the two former drugs under the same condition. It may be noteworthy, however, that the inhibitory effect of conteben on the growth of tubercle bacilli was demonstrated only in the media containing 90 per cent serum collected within 3 hours after injection, nevertheless, the bacteriostatic activity in the serum of the rabbit administered with conteben in above stated dose should be very poor in comparison with the bacteriostatic activity of two former drugs, as the growth of bacilli was observed in the media containing 5 and 10 per cent serum collected even only 30 minutes after injection of conteben.

This fact suggests that conteben remains in the blood relatively longer than the other drugs, and because of this, clinical effects can be obtained with relatively small amount of conteben.

Conclusion

(1) There is not remarkable difference between the growth of tubercle bacilli in Kirchner's medium containing 10 per cent serum and in the medium prepared so as to consist of 90 per cent Kirchner's basic solution concentrated to tenfold.

(2) In the medium containing 90 per cent serum, the bacteriostatic activity of streptomycin is reduced considerably, that of PAS is reduced slightly and that of conteben is almost unchanged.

(3) In the medium containing 90 per cent serum of the animal injected with streptomycin, PAS or conteben, inhibition of growth of tubercle bacilli is observed.

(4) These bacteriostatic actions are maintained for certain periods of time.

References

(1) O. KIRCHNER: Zbl. Bakt., 1932, Bd. 124, Org. S. 403.
(2) DUBOS, R.J., DAVIS, B.D. and NIDDLEBROOK, G.: Am. Rev. Tuberc., 1946, *54*, 204.
(3) OGIHARA: Saikingaku-Zasshi, 1941, No. 542, P. 235.
(4) HIROKI and URABE: Manshu-Igaku-Zasshi, 1933, Vol. 19.
(5) MASUDA: Gunidan-Zasshi, 1942, No. 348.
(6) URABE: Kekkaku, 1949, *24*, P. 226.
(7) WILLSTON and YOUMANS: Am. Rev. Tuberc., 1945, *59*, 336.